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The Effects of Nanoemulsion Film Coatings Containing Essential Oils on the Storage Quality of Sugar Beets (*Beta vulgaris* L.)

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ABSTRACT

This study was carried out under controlled conditions to determine the effects of chitosan-based nanoemulsion film coating formulations, formed using different essential oils, on storage quality of sugar beet roots during storage period. In the study, roots of Conviso Smart (KWS) sugar beet variety were coated with nanoemulsion film formulations containing thyme (*Thymus vulgaris*), clove (*Szygium aromaticum*), ginger (*Zingiber officinale*) and tea tree (*Melaleuca alternifolia*) essential oils doses of 250, 500 and 1000 ppm immediately after harvest. The roots were stored in plastic cases under controlled conditions (+ 8-10°C, 85-90% relative humidity) for 90 days. Weight loss in beet roots was determined at 30-day intervals from the start of the storage period, and at the end of the storage period, firmness, dry matter ratio, brix value, polar sugar, reducing sugar, alpha amino nitrogen and glycine betaine contents and fungal infection developments in beet roots were also evaluated. The film coating applications significantly affected postharvest weight and quality losses in sugar beet roots. The applications significantly reduced roots weight loss during storage compared to the control. Although the polar sugar ratios were higher in film coated roots compared to the control, alpha-amino nitrogen, glycine betaine, and reducing sugar contents showed significant decreases. White mold and green mold infections on the roots were significantly decreased, especially with high dose film coating applications. The highest dry matter ratio, brix values and firmness were obtained from film coatings containing 1000 ppm cinnamon and thyme essential oils. While polar sugar ratio was higher in root which film coated compared to the control, alpha amino nitrogen, glycine betaine and reducing sugar contents showed significant decreases. White mold and green mold infections developing on root showed significant decreases especially with film coating applications applied at high doses. The study concluded that coating sugar beet roots with nanoemulsion film formulations containing essential oils can significantly reduce, weight and quality losses, as well as fungal disease development, during the storage period.

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Introduction

Sugar beet is one of the most important strategic products, serving as a raw material to many industrial sectors. In Türkiye, approximately 23.5 million tons of sugar beet and 3.2 million tons of sugar were produced on about 305 thousand hectares of land in 2023 (Anonim, 2024). Considering the campaign periods and processing capacities of sugar factories, it is evident that the sugar beets produced each year must be stored for certain periods before processing. Sugar beet storage includes all stages from harvest to processing at the factory, and the difference between the amount of beets received at the factory (excluding discarded waste) and amount of processed is referred to as storage loss. The key factors affecting post-harvest storage losses of sugar beets include waiting time at the factory, silo height, climatic conditions, as well as diseases and pests (English, 2020).

Post-harvest quality parameters of root crops are significantly affected by the storage method, duration, and the temperature and humidity of the storage environment. Due to the limited processing capacity of factories, the storage periods of harvested beets are extended. Prolonged storage results in the loss of water, dry matter, and consequently weight, in the root crops. Additionally, environmental factors contribute to the proliferation of fungal and bacterial pathogens in the storage environment, leading to substantial losses in both the quantity and quality of the beets to be processed. In root crops stored for extended periods, the development of fungal pathogens such as *Botrytis cinerea*, *Phoma betae* and *Penicillium vulpinum* is exacerbated by the effects of temperature and humidity. Campbell et al. (2014) reported that fungal and bacterial infections, caused by fluctuations in temperature and humidity under open storage conditions, further

contribute to sucrose losses in root crops. During the storage, it is crucial to maintain the sucrose levels of sugar beets and minimize losses caused by microbial infections. Since the use of chemical fungicides during the storage phase of sugar beets, which supply raw materials for the food industry, is not considered feasible from a health perspective, investigating natural methods to reduce fungal infections and prevent weight and quality losses during the storage is an important topic.

Chitosan, derived from the chitin in crustacean shells, is a natural, edible polymer that is non-toxic and environmentally friendly (Iber et al., 2022). It is a linear polysaccharide produced through the deacetylation of chitin. Chitosan is widely recognized as a versatile biopolymer, commonly used as an edible coating for fruits and vegetables due to its excellent film-forming ability, biocompatibility, and robust mechanical strength (Mesa et al., 2021). Additionally, chitosan possesses various health-promoting functional properties, including antifungal, antibacterial, antitumor, antioxidative, and hypocholesterolemic activities (Naveed et al., 2019). Recent studies have focused on enhancing edible polymeric coatings by incorporating natural additives, significantly boosting their protective properties (Zhang et al., 2023). Extensive research has been conducted on chitosan, particularly in combination with other natural components such as additional natural polymers, polyphenolics, nanoemulsions, and essential oils, to improve its functionality. The incorporation of essential oils into edible coatings has notably enhanced their antimicrobial effectiveness. Studies have shown that essential oils prevent the development of fungal infections that cause economic damage to crops and exhibit strong antifungal activity (Macwan et al., 2016; Nazzaro et al., 2017). It has been reported that by treating sugar beet roots with essential oils of dill (*Anethum graveolens* L.), clove (*Szygium aromaticum*), and Turkish pickling herb (*Echinophora tenuifolia*) after harvest and storing them, weight losses during storage can be reduced by approximately 37% compared to the control (Ok et al., 2022). In related studies, it was found that certain essential oils applied to plant leaves can influence enzyme activities, leading to significant effects on photosynthetic activity, vegetative period, maturation, and disease development (Ok, 2020). In another study, essential oils applied to sugar beet leaves affected sucrose synthesis enzyme activities and reduced the incidence and severity of leaf spot infections (Kurşunatan, 2019).

This study aims to determine the effects of chitosan-based nanoemulsion film coatings, created using essential oils of thyme (*Thymus vulgaris*), clove (*Szygium aromaticum*), ginger (*Zingiber officinale* Rosc.), and tea tree (*Melaleuca alternifolia*) applied at the beginning of storage, on the development of fungal infections, as well as weight and quality losses in sugar beet roots.

Materials and Methods

Materials

The study was conducted in 2022 at the experimental fields and cold storage facilities of Isparta University of Applied Sciences, Faculty of Agriculture. In the study, the seed material used was the Conviso Smart variety obtained from KWS Türk, while the essential oils of *T. vulgaris*, *S. aromaticum*, *Z. officinale* Rosc., and *M. alternifolia* and chitosan were used as application materials.

Methods

Sugar beet production

Beet roots required for the storage study were grown in 2022 at the experimental fields of the Faculty of Agriculture, Isparta University of Applied Sciences. The sowing was done in the first week of April using a pneumatic seeder with row spacing of 10x45 cm. Along with sowing, 40 kg/da of Super Beet fertilizer (N:13-P:18-K:15-S:10) and 18 kg/da of ammonium sulfate (21% N) were applied, and during the first hoeing, 20 kg/da of urea (46% N) was applied, resulting in a total fertilization of 18-7-6 kg/da N-P-K. Irrigation was carried out using a sprinkler system when the soil moisture fell below 50% or when the top 10 cm of soil dried out. After the completion of plant emergence, the plants were thinned to a row spacing of 20 cm during the first hoeing. Betanal maxxPro (47 g/L Desmedipham + 75 g/L Ethofumesate + 27 g/L Lenacil + 60 g/L Phenmedipham, Bayer CropScience AG) herbicide was used when the sugar beets had 2-6 leaves, and two manual weeding sessions were performed during the vegetation period for control of weeds. After the 170-day vegetation period (October 20), the sugar beets were harvested using a beet fork, and their tops were cut off. From the harvested beets, those with an average weight of 1250-1500 g, no branching, and free from diseases and pests were selected for the storage study, and the storage process began on the same day.

Extraction of essential oil and GC-FID/GC-MS analysis

In the study, essential oils of *Z. officinale* Rosc. and *M. alternifolia* were purchased, while the essential oils of *T. vulgaris* and *S. aromaticum* were obtained through Clevenger-type hydro-distillation. Each plant species was distilled in a boiling flask of the distillation device at 100°C for 3 hours to obtain the essential oils. The components of the purchased and hydro-distilled essential oils were determined using a GC/MS (Gas Chromatography/Mass Spectrometry) system (QP-5050 GC/MS with a quadrupole detector) at the SDU Experimental and Observational Research and Application Center. The GC/MS conditions were as follows: Capillary column: CP-Wax 52 CB (50 m × 0.32 mm, 0.25 μm); Oven temperature program: increased by 10°C per minute from 60°C to 220°C, with a 10-minute hold at 220°C; Total run time: 60 minutes; Injector temperature: 240°C; Detector temperature: 250°C; Carrier gas: Helium (20 ml/min). The major components of the essential oils were identified as follows: *Z. officinale*: 28.94% Sesquithujene, 10.42% Camphene; *M. alternifolia*: 21.64% α-pinene, 21.09% φ-terpinene; *T. vulgaris*: 44.11% Thymol, 23.26% Cymol; *S. aromaticum*: 82.4% Eugenol, 8.6% Eugenol acetate.

Application of essential oil film formulations

Chitosan-based nanoemulsion film formulations containing essential oils were prepared by mixing the essential oils into a 0.5% chitosan solution. For this purpose, four different essential oils were prepared at concentrations of 250, 500, and 1000 ppm using Tween-80, and then added to a 0.5% chitosan solution. The mixture was stirred for 1 hour at 25,000 rpm to obtain a

homogeneous mixture. Thus, chitosan-based nanoemulsion (oil-in-water (o/w)) film formulations containing essential oils at different concentrations were created. Root stalks that underwent no treatment and beet roots coated only with chitosan were evaluated as controls. In the storage study, 30 beet roots with an average weight of 1250-1500 g were stored in plastic crates in a cold storage (+8-10°C) for 3 months, with 3 replications for each treatment. Prior to the storage period, the chitosan-based nanoemulsion film coating formulations prepared with essential oils were sprayed onto the entire surface of the roots using 100 ml handheld sprayers. Roots with no treatment were stored as the control group. Throughout the storage period, weight loss in the roots was measured at 30-day intervals. At the end of the storage period, parameters such as firmness, dry matter content, Brix value, polar sugars, reducing sugars, alpha-amino nitrogen, and glycine betaine content were determined, and fungal infections in the root stalks were monitored.

During the storage period, the beet roots were weighed every 30 days, and weight loss was expressed as a percentage relative to the initial weight (AOAC, 1994). Polar sugar and α-amino nitrogen analyses were performed according to the ICUMSA (International Commission for Uniform Methods of Sugar Analysis) methods. The polar sugar content was determined using a polarimetric method based on cold digestion (Mc Ginnis, 1982), while the α-amino nitrogen content was determined using the bluenumber method (Kubadinow & Wieninger, 1972) by spectrophotometric analysis. The Brix value of the root stalks was determined by measuring the refractive index of the juice after cooling the beet extract at 20°C, without any further treatment, and expressing it as % dry matter (Kavas & Leblebici, 2004). Reducing sugar content was determined following the method of Honda et al. (1980) and the results were expressed in mg/100 g fresh weight. The growth of white and green mold on the beet roots were determined by measuring the infection diameter in millimeters on 5 randomly selected infected beet roots.

Statistical Analysis

The data obtained from the study were analyzed using the GLM procedure of the SAS (2009) statistical software package, applying standard variance analysis technique (ANOVA). Differences between means were determined using the LSD test.

Results and Discussion

Weight Loss (%)

The effects of the treatments and storage duration on the weight loss of sugar beet roots were statistically significant ($P<0.01$). In the study, all essential oil film formulations, except for all doses of *Z. officinale* (8.06-8.46%) film formulations, reduced weight loss in the beets compared to the control (8.91%). The lowest weight loss was observed with the *M. alternifolia* 250 ppm (5.67%) and 500 ppm (5.18%) film formulations. During the storage process, root weight losses increased significantly (Table 1). The high water content and metabolic activity of sugar beet roots lead to weight and quality losses after harvest. The resulting weight losses vary depending on the variety, injury status, the physiological maturity of the root, disease condition, storage temperature, humidity, and duration (Ada, 2010; Cirit et al., 2019). The optimal storage temperature for beets is between 4-6°C, with relative humidity between 95-98%. High temperatures and low humidity conditions increase weight losses. In this study, some essential oil film coating applications showed a reducing effect on weight loss compared to the control group, which had no treatment. The reduced weight loss observed with the *M. alternifolia* film formulation is believed to be due to this treatment's ability to reduce infection development in the roots. In related studies, it has been reported that as the storage period increases in open storage, weight loss also increases (Kenter & Hoffmann, 2009). Furthermore, it has been found that weight losses are higher at the beginning of the storage period, with an average daily weight loss of 2.08% for piles stored for 5-7 days in Turkey (Ada, 2010). Can Çetin (2012) reported that coating pomegranate seeds (*Punica granatum*) with a 1% chitosan solution reduced weight loss. Who's results also showed that chitosan coating increased the shelf life of pomegranate seeds stored at refrigerator temperatures from 15 days to 19 days. Zhang et al. (2012) reported that, in comparison with the control samples, the chitosan-coated samples were able to control the respiration process by forming a barrier on the sample surface, thus preventing the exchange of O_2 and CO_2 between the coated layers of the sample and the environment, thereby extending the shelf life.

Firmness (N)

The effect of treatments on beet roots firmness was statistically significant ($P<0.05$). At the end of the storage period, the root firmness was higher in the applications with *M. alternifolia* oil at 250 and 500 ppm doses (88.3-89.0 N) and *T. vulgaris* oil film formulations (86.3-88.7 N). In contrast, the firmness of beets treated with *Z. officinale* oil formulations at all doses (74.7-76.6 N) was similar to the control (73.3 N) (Table 2). Firmness, a response to physical pressure, is related to the turgor of the beet root, and to prevent the loss of firmness during storage, water loss needs to be minimized. However, during the storage period, sugar beets continuously lose moisture, and the loss of moisture increases as a result of temperature fluctuations and the development of infections. Several studies have reported that in tuber crops, firmness decreases with sprouting, disease development, and moisture loss, and losses become more pronounced when storage temperatures exceed 10-12°C (Nedomová et al., 2016). Chitosan coating can act as a protective barrier, reduce oxygen permeability, and consequently delay respiration and fruit ripening (Yang & Ge 2021; Alpos & Bayogan 2023). The addition of essential oil edible polymers improves the barrier properties, creating a beneficial microclimate on the surface of the processed samples. This reduces moisture loss, respiration rates, and effectively inhibits the increase in ethylene production in the processed samples.

Table 1. Change in weight loss in sugar beets during storage period (%)

Applications	30 day	60 day	90 day	Mean
Z. officinale 250 ppm	4.53	8.33	11.3	8.07 ^{ac}
Z. officinale 500 ppm	4.27	8.17	11.7	8.06 ^{ac}
Z. officinale 1000 ppm	4.30	8.40	12.7	8.46 ^{ab}
S. aromaticum 250 ppm	3.36	7.80	9.43	6.87 ^{eg}
S. aromaticum 500 ppm	3.93	8.17	10.1	7.40 ^{ce}
S. aromaticum 1000 ppm	4.13	8.47	10.7	7.76 ^{bd}
T. vulgaris 250 ppm	3.37	7.27	9.33	6.66 ^{eg}
T. vulgaris 500 ppm	3.13	6.90	9.10	6.38 ^{fh}
T. vulgaris 1000 ppm	3.70	7.50	9.93	7.04 ^{df}
M. alternifolia 250 ppm	2.73	6.43	7.83	5.67 ^{hı}
M. alternifolia 500 ppm	2.53	5.80	7.20	5.18 ^{ı}
M. alternifolia 1000 ppm	3.23	6.87	8.23	6.11 ^{gh}
Chitosan	3.67	7.60	10.1	7.13 ^{df}
Control	4.93	8.70	13.1	8.91 ^{a}
Mean	3.70 ^{c}	7.60 ^{b}	10.1 ^{a}	
CV (%)	13.0			
Lsd_{int}: 1.50				

Table 2. Firmness (N), dry matter ratio (%) and brix (%) changes in sugar beets during the storage period

Applications	Mean		
	Firmness (N)	Dry matter ratio (%)	Brix (%)
Z. officinale 250 ppm	76.6 ^{dg}	21.8 ^{a}	26.7 ^{ab}
Z. officinale 500 ppm	74.7 ^{fg}	21.9 ^{a}	27.8 ^{a}
Z. officinale 1000 ppm	75.3 ^{eg}	21.3 ^{ab}	27.8 ^{a}
S. aromaticum 250 ppm	81.3 ^{cd}	20.3 ^{bc}	27.4 ^{ab}
S. aromaticum 500 ppm	79.7 ^{ce}	20.3 ^{bc}	26.4 ^{ab}
S. aromaticum 1000 ppm	83.3 ^{bc}	20.5 ^{b}	26.9 ^{ab}
T. vulgaris 250 ppm	86.3 ^{ab}	18.7 ^{d}	22.5 ^{de}
T. vulgaris 500 ppm	86.3 ^{ab}	18.8 ^{d}	23.6 ^{cd}
T. vulgaris 1000 ppm	88.7 ^{a}	18.6 ^{d}	22.6 ^{de}
M. alternifolia 250 ppm	89.0 ^{a}	18.6 ^{d}	22.3 ^{de}
M. alternifolia 500 ppm	88.3 ^{a}	19.2 ^{cd}	22.8 ^{de}
M. alternifolia 1000 ppm	83.3 ^{bc}	18.9 ^{d}	21.6 ^{e}
Chitosan	78.3 ^{df}	20.3 ^{bc}	24.4 ^{c}
Control	73.3 ^{g}	22.2 ^{a}	26.1 ^{b}
Lsd	4.92	1.11	1.43
CV (%)	3.60	4.80	3.43

Dry Matter Content (%)

The changes in beet root dry matter content during storage were found to be statistically significant (P<0.01) based on the treatments. At the end of the storage period, the dry matter content of the beet roots treated with *Z. officinale* oil film formulations all doses (21.3–21.9%) was similar to the control (22.2%). In contrast, beet roots treated with *T. vulgaris* oil (18.6–18.8%) and *M. alternifolia* oil (18.6–19.2%) film formulations exhibited lower dry matter content compared to the control (Table 2). During storage, the loss of moisture from the beet roots led to a relative increase in dry matter content. In sugar beets, the outer layers of the beet root lose water quickly after harvest, with the loss being most significant in the initial days and gradually decreasing thereafter (Ada, 2010). Several studies on this topic have also reported a relative increase in dry matter content during the storage period (Demirel & Akınerdem, 2016). It is believed that the application of ginger essential oil film coatings causes phytotoxicity in the beet roots, leading to increased respiration and consequently greater moisture loss and an increase in dry matter content. Beet roots that were not coated with chitosan are thought to have lost more moisture compared to the chitosan-coated ones, resulting in a proportional increase in dry matter content. Reportedly, the chitosan coating forms a barrier on the sample surface, preventing the exchange of O_2 and CO_2 between the coated layers of the sample, thereby controlling respiration-induced transpiration levels, delaying crop dehydration, and reducing surface shriveling (Zhang et al., 2012).

Brix (%)

The changes in beet root Brix values during storage were found to be statistically significant (P<0.01) based on the treatments. At the end of the storage period, the Brix value of the beets treated with *Z. officinale* oil (26.7–27.8%) and *S. aromaticum* oil (26.4–27.4%) film formulations was higher than the control (26.1%), while Brix values of beets treated with *T. vulgaris* oil 250 ppm and 1000 ppm (22.5-22.7%) and *M. alternifolia* oil (21.6–22.8%) film formulations were lower than the control (Table 2). The Brix value indicates the amount of dissolved solids (mainly sugars) in water, and as the moisture content

of the beet roots decreases, the Brix value increases. During storage, the loss of moisture in the beet roots led to an increase in the Brix values. Similar results were also observed for the dry matter content of the beet roots. Babaee et al. (2013) reported that when the moisture content of beet roots decreased from 72.5% to 70%, the Brix value increased from 19.10% to 24.90%. Hirschmüller and Krocher (1968) also reported that the dry matter and Brix values increased due to moisture loss in beet root. In our study, similar results were observed, with beet roots treated with *Z. officinale* and *S. aromaticum* oil film formulations showing a proportional increase in Brix values. This is likely due to the increased cell permeability, which leads to a rise in dry matter content.

Polar Sugar Content (%)

The changes in the polar sugar content of beet roots during storage were found to be statistically significant ($P<0.01$) based on the treatments. The polar sugar content in beets treated with all doses of *Z. officinale* (18.9–19.7%) and *S. aromaticum* (18.8–19.9%), and *T. vulgaris* at 250 and 500 ppm (18.6%) increased compared to the control, while the polar sugar content in the beets treated with *M. alternifolia* oil (17.1–17.7%) at all doses and *T. vulgaris* at 1000 ppm (18.0%) were similar to control (17.1%) (Table 3). During storage, as the beet roots lost moisture, their dry matter content increased. The sugar content in the beet roots constitutes a significant portion of the dry matter, so as the dry matter content increases, the sugar content also increases proportionally. Therefore, it is expected that treatments that increase the dry matter content will also increase the polar sugar content. Indeed, the treatments that resulted in the highest dry matter content also showed the highest polar sugar content. Ok et al. (2022) reported that the application of 1000 ppm *S. aromaticum* essential oil at the beginnig of storage in sugar beet beet roots decreased the polar sugar content by 17.1%. Furthermore, it has been noted that the activities of enzymes involved in sucrose synthesis, such as sucrose phosphate synthase and sucrose synthase, generally increase with essential oil film coatings, with these increases reaching up to 3-4 times. These essential oils may alter the polar sugar content by affecting the activities of sucrose phosphate synthase, sucrose synthase, and invertase enzymes (Kurşunatan, 2019).

α-Amino Nitrogen Content (mg/100g)

Changes in the α-amino nitrogen content of beet roots during storage were found to be statistically significant ($P<0.01$) based on the treatments applied at the beginning of storage. At the end of the storage period, the lowest α-amino nitrogen content was observed in the beet roots treated with *M. alternifolia* oil 250 ppm (6.70 mg/100g) and 500 ppm (5.67 mg/100g) film formulations. On the other hand, the α-amino nitrogen content of beet roots treated with *Z. officinale* (9.47–10.6 mg/100g) and *S. aromaticum* 250 ppm (9.80 mg/100g) and 500 ppm (9.87 mg/100g) film formulations were similar to control (10.6 mg/100g) (Table 3). In sugar beets, the hydrolysis of insoluble nitrogen compounds like proteins into amino acids results in an increase in α-amino nitrogen concentration (Vukov & Hangyál, 1985). In sugar beets, α-amino nitrogen interferes with the crystallization of sugar, thereby reducing refined sugar yield. It is believed that the increase in the α-amino nitrogen content of beet roots is related to the proportional increase in dry matter content. Under unsuitable storage conditions, both direct sugar losses due to respiration and indirect losses from the accumulation of non-sugar substances that reduce white sugar yield have been reported (Van der Poel et al., 1998). Another factor affecting α-amino nitrogen content is stress conditions. It has been shown that α-amino nitrogen content increases under mechanical injuries and environmental stress conditions after harvest, with a close, linear relationship between stress and α-amino nitrogen (Sadeghian et al., 2004). The reduction in α-amino nitrogen content in beet roots treated with 250 ppm and 500 ppm *M. alternifolia* essential oil film coatings might be due to the higher stress tolerance of these tubers, such as resistance to storage diseases. Indeed, it has been reported that some terpenoids increase stress tolerance (Mazid et al., 2011).

Table 3. Changes in polar sugar (%) and α-amino nitrogen content (mg/100g) in sugar beets during the storage period

Applications	Mean	
	Polar sugar (%)	α-amino nitrogen content (mg/100g)
Z. officinale 250 ppm	19.4 ^{ab}	9.47 ^{ab}
Z. officinale 500 ppm	18.9 ^{ad}	10.4 ^{a}
Z. officinale 1000 ppm	19.7 ^{a}	10.6 ^{a}
S. aromaticum 250 ppm	19.1 ^{ac}	9.80 ^{ab}
S. aromaticum 500 ppm	18.8 ^{ad}	9.87 ^{ab}
S. aromaticum 1000 ppm	19.9 ^{a}	8.13 ^{cd}
T. vulgaris 250 ppm	18.6 ^{af}	9.10 ^{bc}
T. vulgaris 500 ppm	18.6 ^{ad}	7.77 ^{de}
T. vulgaris 1000 ppm	18.0 ^{bg}	7.30 ^{de}
M. alternifolia 250 ppm	17.7 ^{cg}	6.70 ^{ef}
M. alternifolia 500 ppm	17.5 ^{dg}	5.67 ^{f}
M. alternifolia 1000 ppm	17.1 ^{fg}	7.47 ^{de}
Chitosan	17.2 ^{eg}	8.00 ^{cd}
Control	17.1 ^{g}	10.6 ^{a}
Lsd	1.46	1.24
CV (%)	4.73	8.59

Table 4. Changes in reducing sugar ratio (mg/100g) and glycine betaine (mg/g fw) in sugar beets during storage

Applications	Mean	
	Reducing sugar ratio (mg/100g)	Glycine betaine content (mg/g fw)
Z. officinale 250 ppm	0.35 [bd]	4.07 [b]
Z. officinale 500 ppm	0.46 [ab]	5.02 [a]
Z. officinale 1000 ppm	0.43 [ac]	4.72 [a]
S. aromaticum 250 ppm	0.33 [ce]	3.83 [bc]
S. aromaticum 500 ppm	0.47 [a]	5.25 [a]
S. aromaticum 1000 ppm	0.42 [ac]	5.04 [a]
T. vulgaris 250 ppm	0.27 [de]	3.22 [de]
T. vulgaris 500 ppm	0.29 [de]	3.27 [cd]
T. vulgaris 1000 ppm	0.26 [de]	3.00 [de]
M. alternifolia 250 ppm	0.24 [e]	2.67 [e]
M. alternifolia 500 ppm	0.26 [de]	2.66 [e]
M. alternifolia 1000 ppm	0.25 [de]	3.22 [de]
Chitosan	0.31 [de]	2.76 [de]
Control	0.48 [a]	5.15 [a]
Lsd	0,11	0,58
CV%	18.7	9.0

Reducing Sugar Content (mg/100g)

Changes in the reducing sugar content of beet roots during storage were found to be statistically significant (P<0.01) based on the treatments applied at the beginning of storage. In the study, the reducing sugar content was lower in the beet roots treated with all doses of *M. alternifolia* oil (0.24–0.26 mg/100g) and *T. vulgaris* (0.26–0.29 mg/100g) film formulations, as well as those treated with chitosan (0.31 mg/100g) and *S. aromaticum* 250 ppm (0.33 mg/100g) film formulations, compared to the control (0.48 mg/100g). However, the reducing sugar content of beet roots treated with *Z. officinale* 500 and 1000 ppm (0.46–0.43 mg/100g) and *S. aromaticum* 500 and 1000 ppm (0.47–0.42 mg/100g) film formulations was similar to the control (Table 4). During the storage period, sucrose is hydrolyzed into reducing sugars through the action of invertase, an enzyme involved in respiration, leading to an increase in reducing sugar content (Zrenner et al., 1996). In line with this, the high doses of *Z. officinale* and *S. aromaticum* essential oils in our study are thought to have caused phytotoxicity, increasing the respiration rate and consequently contributing to the increase in reducing sugars as sugars were broken down for respiration. Previous studies have also reported that some essential oils, such as dill and clove, at high doses, cause phytotoxicity by increasing respiration rates, leading to the breakdown of sugars and an increase in reducing sugars (Ok et al., 2022).

Glycine Betaine Content (mg/g fw)

Changes in the glycine betaine content of beet roots during storage were found to be statistically significant (P<0.01) based on the treatments applied at the beginning of storage. In the study, the glycine betaine content was lower in beet roots treated with all doses of *M. alternifolia* oil (2.66–3.22 mg/g fw) and *T. vulgaris* oil (3.00–3.27 mg/g fw) film formulations, as well as those treated with chitosan (2.76 mg/g fw), compared to the control (5.15 mg/g fw). However, the glycine betaine content of beet roots treated with *Z. officinale* at 500 and 1000 ppm (5.02–4.72 mg/g fw) and *S. aromaticum* at 500 and 1000 ppm (5.25–5.04 mg/g fw) film formulations was similar to the control (Table 4). The increase in glycine betaine content in plants treated with essential oils can be attributed to the ability of these oils to influence certain biochemical processes, such as promoting the synthesis of phytoalexins (which are responsible for stress resistance in some plants). Indeed, the increase in glycine betaine synthesis under stress conditions suggests that this compound plays an active role in enhancing stress tolerance. Glycine betaine functions as a methyl donor in some biochemical synthesis pathways, thereby increasing tolerance to stress (Pummer et al., 2000).

White and Green Mold Diameter (cm)

The changes in the rate of green mold formation on the root crops were statistically significant (P<0.01) depending on the treatments applied at the beginning of storage. All film coating treatments reduced the diameter of green mold infection on the root crops compared to the control group (9.0%), with the highest antifungal activity observed in the *M. alternifolia* oil (3.30-4.03%) film formulations at all doses, as well as *T. vulgaris* oil at 500 ppm (3.83%) and 1000 ppm (4.00%) applied to the root crops (Table 5).

The changes in the rate of white mold formation on root crops were statistically significant (P<0.01) depending on the treatments applied at the beginning of storage. The diameter of white mold infection on the root crops was reduced by all doses of *M. alternifolia* oil (1.63-2.03%) and *T. vulgaris* oil (2.13-2.30%) film formulations, as well as chitosan (1.87%) treatments, compared to the control (3.07%). However, all doses of *Z. officinale* (3.23-3.40%) film formulations, as well as *S. aromaticum* oil at 1000 ppm (2.73%) and 500 ppm (2.83%), showed similar or higher levels of white mold development compared to the control (Table 5).

Respiration of sugar beets stored in high piles, especially in the middle sections of the piles, leads to an increase in temperature. Combined with environmental factors, this creates a favorable environment for the development of disease pathogens.

Table 5. Changes in white mold diameter (cm) and green mold diameter (cm) in sugar beets during storage period

Applications	Mean	
	Green mold diameter (cm)	White mold diameter (cm)
Z. officinale 250 ppm	6.60 [b]	3.23 [a]
Z. officinale 500 ppm	6.17 [b]	3.27 [a]
Z. officinale 1000 ppm	6.10 [b]	3.40 [a]
S. aromaticum 250 ppm	5.07 [c]	2.73 [ad]
S. aromaticum 500 ppm	4.20 [de]	2.83 [ac]
S. aromaticum 1000 ppm	4.10 [de]	2.40 [be]
T. vulgaris 250 ppm	4.10 [de]	2.13 [cf]
T. vulgaris 500 ppm	3.83 [ef]	2.30 [cf]
T. vulgaris 1000 ppm	4.00 [df]	2.13 [cf]
M. alternifolia 250 ppm	4.03 [df]	1.83 [ef]
M. alternifolia 500 ppm	3.30 [f]	1.63 [f]
M. alternifolia 1000 ppm	4.00 [df]	2.03 [df]
Chitosan	4.70 [cd]	1.87 [ef]
Control	9.0 [a]	3.07 [ab]
Lsd	0.78	0.70
CV%	9.44	16.9

It has been reported that pathogens transmitted from the field cause diseases under unsuitable storage conditions, with the most common diseases observed in storage being gray mold (*Botrytis cinerea*), black rot (*Alternaria radicina*), bacterial soft rot (*Pectobacterium caratovora*), blue-green mold (*Penicillium* spp.), white rot (*Sclerotinia sclerotiorum*), and soft rot (*Rhizopus oryzae*) (Tülek & Dolar, 2011). Chitosan has antimicrobial activity against bacteria, yeast, and fungi. Chitosan is considered a soluble chelating agent and activator because of the positive charge on C-2 of the glucosamine monomer. These characteristics provide good antimicrobial activity. The destruction of protein and intercellular components occurs due to the interaction between the amine groups in the positively charged chitosan molecule and the negatively charged microbial cell membrane (Goy et al. 2016). Secondary metabolites in plants play protective roles, such as antioxidant activity, scavenging free radicals, and absorbing UV rays. In addition to these, they also form part of the plant's defense mechanism against microorganisms (Kennedy & Wightman, 2011). It has been reported by Mazaro et al. (2008) that the application of essential oils and certain plant extracts increases the production of phytoalexins in plants. In fact, the essential oil film formulations of *M. alternifolia* and *T. vulgaris* used in this study demonstrated high antifungal activity against gray mold and green mold infections. Similarly, Cheng and Shao (2011) in their work, demonstrated the antifungal activity of *M. alternifolia* essential oil against *Penicillium species*. Total inhibition of mycelial growth of both *Penicillium* species was achieved with 2.75% of this essential oil. In this context, Zhang et al. (2018) reported the antifungal effect of *M. alternifolia* essential oil by inhibiting the growth of *P. italicum* and *P. digitatum*. The results obtained by Yonghua et al. (2017) showed that *M. alternifolia* essential oil exhibits antifungal activity against *P. expansum*. Can Çetin (2012) found that microbial spoilage was delayed in pomegranate arils coated with chitosan.

Conclusion

The study found that by coating sugar beet roots with essential oil film formulations after harvest and storing them, weight losses during the storage period could be reduced by approximately 42% compared with the control. Essential oil film coating treatments had a significant impact on the storage quality of the roots, and the contents of α-amino nitrogen and reducing sugars, which negatively affect quality, were significantly reduced by some treatments. High levels of phytotoxicity occurred in roots treated with the *Z. officinale* essential oil film formulation. The *M. alternifolia* and *T. vulgaris* essential oil film formulations significantly reduced the development of gray mold and green mold infections in the roots. It was found that treating sugar beet roots before storage, especially with a 500 ppm *M. alternifolia* essential oil film formulation, could significantly reduce both post-harvest weight and quality losses as well as fungal pathogen development.

Declarations

This study was presented at the 7th International Anatolian Agriculture, Food, Environment and Biology Congress, (Kastamonu, TARGID 2024)

Author Contributions

All authors (F.Z.O. and A.S.) contributed to the writing—original draft, formal analysis, review and editing, data curation, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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